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13. ABSTRACT (Maximum 200 Words)

The purpose of these studies is to elucidate the pharmacogenetic factors that contribute to variation in human response to tamoxifen (TAM) and raloxifene (RAL). We had previously identified and partially characterized common genetic polymorphisms in two human drug-metabolizing genes, SULT1A1 and UGT1A1. We hypothesized that these polymorphisms contributed to variation in TAM or RAL metabolism. These studies were divided into three aims with the purpose of 1) biochemically characterizing the contribution of these enzymes to the metabolism of TAM and RAL; 2) developing cell model systems to study allele-specific differences in cellular response to these molecules and; 3) perform a clinical pharmacogenetic study to evaluate the association of common genetic polymorphisms in drug metabolizing genes with variable clinical response to TAM. Thus far we have determined that SULT1A1 and UGT1A6 contributed to the inactivation of 4-hydroxytamoxifen (OHT), the active metabolite of TAM, and that a separate enzyme, UGT1A9 catalyzed the glucuronidation of RAL. We established MCF-7 breast cancer cell lines stably expressing the wildtype and variant SULT1A1 alleles and have measured allele-specific differences in the response of these cells to estrogens and OHT. These studies suggest that pharmacogenetic factors might contribute to variable cellular response to antiestrogens.

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Pharmacogenetic Factors Contributing to Variation in Response to Tamoxifen and Raloxifene

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INTRODUCTION

The goals of this proposal are to elucidate the pharmacogenetic factors that influence cellular response to tamoxifen (TAM) and raloxifene (RAL). Specifically, the work described represents a stepwise approach to the study of genetic polymorphisms in human sulfotransferase (SULT) and UDP-glucuronosyltransferase (UGT) genes. Those studies will progress from basic biochemical studies to the use of cell models and will culminate in a clinical pharmacogenetic study. In the first aim, we proposed to biochemically characterize the capacity of wildtype and variant SULT1A1, SULT1A2 and UGT1A6 proteins to conjugate 4-hydroxytamoxifen (OHT) and raloxifene RAL. The second aim focussed on the development of cell models to study allele-specific differences in cellular response to these antiestrogens. Finally, the third aim will determine the association of genetic polymorphisms in several metabolic pathways with human response to TAM in a clinical setting.

BODY

Specific Aim1. Biochemically characterize the capacity of recombinant wildtype and variant SULT1A1, SULT1A2 and UGT1A6 proteins to conjugate OHT and RAL in _in vitro_ assays. We proposed four sub aims associated with this aim—each to be completed within the first year of funding. Those included:

1) Generation of recombinant wildtype and variant UGT1A6 allozymes
2) Biochemical characterization of the SULT1A1 and SULT1A2 allozymes with regard to their capacity to sulfate OHT and RAL.
3) Optimization of the glucuronidation assay.
4) Biochemical characterization of the UGT1A6 allozymes with regard to their capacity to glucuronidate OHT and RAL.

Thus far we have completed the biochemical characterization of SULT1A1 and SULT1A2 allozymes. SULT1A1 catalyzes the sulfation of OHT with an apparent Km value of 20 μM (Figure 1). The kinetics of that reaction are shown in figure 1 along with the kinetics for 17β-estradiol (E2) for comparison. The rate of sulfation of OHT is higher than the competing reaction with E2 at physiological concentrations of both substrates. Furthermore we also evaluated the capacity of two additional SULTs to sulfate OHT—SULT1A2 and SULT1E1 (an isoform with high affinity toward E2). Both of those enzymes exhibited minimal capacity to sulfate OHT. None of the SULT isoforms (including SULT1A1) were capable of sulfating raloxifene as measured in a standard radiometric SULT assay. This was not surprising as raloxifene is known to be excreted almost exclusively as glucuronidated conjugates. Therefore, we next evaluated the capacity of wildtype UGT1A1, UGT1A6 and UGT1A9 to glucuronidate OHT and RAL. UGT1A6 and UGT1A9 both contributed to the glucuronidation of OHT while only UGT1A9 glucuronidated RAL. Those data have become the basis of a DOD Breast Concept Award to study genetic polymorphisms in the human UGT1A9 gene (BC995705).

We have experienced difficulties expressing recombinant UGT1A6 allozymes in our baculovirus system. However, consultation with Dr. Brian Burchell at the University of Dundee (Dundee, Scotland) has led us to develop a mammalian cell system (V79 Chinese hamster lung cells) in which we will express and purify recombinant UGT1A6 protein. Once those reagents are generated we will evaluate the biochemical properties of these allozymes with regard to capacity to glucuronidate OHT.

The results of these studies thus far have allowed us to focus on SULT1A1 as the primary SULT isoform contributing to the sulfation of OHT and UGT1A6 contributing to OHT glucuronidation. Furthermore these studies indicate that apparently no SULTs nor UGT1A6 contribute to the conjugation of RAL. Therefore, in Specific Aim 2 we will focus on SULT1A1 and UGT1A6 mediated abrogation of cellular response to OHT (and not RAL).

Specific Aim 2. Determine the antiestrogen response to OHT and RAL of cells in which wildtype and variant SULT1A1 and UGT1A6 allozymes have been expressed. These studies were designed to evaluate whether allele-specific differences in the response of cells to estrogens and antiestrogens could be measured. Sulfation of E2 and OHT results in inactive molecules (ie., they do not interact with the estrogen receptor). Therefore, we hypothesized that cells expressing the less active SULT1A1*2 allozyme would exhibit an enhanced proliferative response to E2 and OHT because those cells would have lower capacity to inactivate those molecules. There were originally six sub aims associated with this specific aim to be completed in months 9 through 30 of the funding period. Those were:

1) Generation of expression constructs
2) Generation of transformed yeast cells
3) ß-galactosidase assays
4) Generation of stably transfected human MCF-7 cell lines
5) Cell proliferation assays
6) Quantitative RT-PCR of stably transfected MCF-7 cell lines

Significant progress has made toward these subaims. MCF-7 cell lines stably expressing SULT1A1*1 and *2 allozymes (as well as a sham transfected pCR3.1cell line) have been generated and clones were selected for further study based upon matched genomic copy number and level of mRNA expression (Figure 2). Those clones were then evaluated for proliferative response to E2 and OHT using standard cell proliferation assays (alamarBLUE). Results indicated that, as hypothesized, the cells expressing SULT1A1*2 exhibited greater proliferative response to E2 and OHT (Figure 3). This was presumably because these cells had lower capacity to inactivate E2 and OHT.

We are currently developing cell lines stably expressing UGT1A6 allozymes and will perform similar proliferative studies with those cells. Based upon the advice of the original reviewers of this grant proposal, we have eliminated the yeast studies as they were largely redundant to the mammalian cell studies. Although not initially a part of these aims--we have also characterized population frequencies of the UGT1A6 polymorphisms (Table 1) and determined genotype/phenotype correlation in a bank of human liver samples from Caucasians with liver cancers. Those studies utilized two relatively specific substrates for UGT1A6 (p-nitrophenol and β–napthol). Overall we measured a 25-fold variation in the rate of glucuronidation among this group of 65 liver samples. Figure 4 illustrates the genotype/phenotype correlation that was observed. Specimens expressing the UGT1A6*2 allele homozygously exhibited a significantly higher rate of glucuronidation.

Collectively these studies suggest that cells expressing SULT1A1*2 exhibited a greater response to E2 and OHT and that genetic polymorphisms within the human UGT1A6 gene might be functionally significant.

Specific Aim 3. Determine the association of SULT1A1, SULT1A2, UGT1A6, CYP3A4 and CYP2D6 genotypes with the clinical response of women who are being prescribed TAM. The purpose of this aim is to determine the pharmacogenetic factors that impact clinical response to TAM. Because TAM is subject to several competing metabolic pathways—each of them polymorphic—we expanded the pharmacogenetic scope in this aim to encompass the oxidative metabolic pathways that activate TAM to OHT (CYP3A4 and CYP2D6). The accrual of patients was to begin in month 6, however we did not receive approval of our clinical protocol from the DOD until July 2001. We are prepared to begin accruing patients in the month of August. We anticipate extending the clinical study by six months to allow for adequate follow-up time. Thus far, we have established a high throughput genotyping assay for SULT1A1 and UGT1A6 using a novel "pyrosequencing" genotyping technology (Pyrosequencing, Inc., Westborough, MA). We will also use this technology to genotype CYP2D6 and CYP3A4 alleles in our clinical studies.

KEY RESEARCH ACCOMPLISHMENTS

- Determined which SULTs and UGTs contribute to conjugation of TAM and RAL.

- Evaluated population frequencies and genotype/phenotype correlation for UGT1A6 alleles.

- Developed a cell model for the study of SULT1A1 allele-specific cellular responses.

- Observed SULT1A1 allele-specific proliferative responses to E2 and OHT.

- Developed high throughput assays for genotyping SULT1A1 and UGT1A6.

REPORTABLE OUTCOMES

Abstracts
UGT1A6 Genetic Polymorphisms: Identification and Genotype/Phenotype Analysis from Human Liver Tissues, J.J. Zalatoris and R.B. Raftogianis, *Clin. Pharm. Ther.*, **69**:77, 2001. Presented (Poster) at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, March, 2001, Orlando, Fla.

Human SULT1A1 Pharmacogenetics: Association of SULT1A1*2 with Heightened Cellular Response to Estrogens and Antiestrogens, R.B. Raftogianis and S.E. Walther, *Clin. Pharm. Ther.*, **69**:94, 2001. Presented (Oral) at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, March, 2001, Orlando, Fla.

Human Phenol Sulfotransferase (SULT1A1) Pharmacogenetics and Regulation of Cellular Response to 17-Estradiol and 4-Hydroxytamoxifen. Rebecca B. Raftogianis and Susan E. Walther, *Proc. Am. Assoc. Cancer Res.*, **42**:266, 2001. Presented (Oral) at the 2001 Annual Meeting of the American Association for Cancer Research, March, 2001, New Orleans, LA.

Invited Symposia

Functional Analysis of Two Human Cytosolic Sulfotransferases (SULT4A1 and SULT1A1), Gordon Research Conference in Drug Metabolism, Plymouth, NH, July, 2001.

Manuscripts

Jeffrey J. Zalatoris and Rebecca B. Raftogianis, Identification of Genetic Polymorphisms in the Human UGT1A6 Gene and Genotype/Phenotype Correlation in Human Liver Tissue. *In preparation.*

CONCLUSIONS

In summary, we have made significant progress in achieving the aims set forth in this grant. Specifically we have determined the SULT1A1 and UGT1A6 contribute to the conjugative metabolism of OHT, the active metabolite of TAM and that UGT1A9 is predominantly responsible for RAL glucuronidation. Furthermore, we have identified and determined the allele frequencies for four common UGT1A6 alleles in ethnically defined human populations and have determined genotype/phenotype relationships for those alleles in a bank of human liver tissues. We have developed a cell model system to study SULT1A1 allele-specific cellular phenotypes and have implemented that system to identify allele-specific proliferative response of cells to estrogens and antiestrogens. Specifically, we have determined that cells expressing the SULT1A1*2 variant respond to those compounds significantly more than cells expressing the SULT1A1*1 allele. Approximately 30% of Caucasians and African Americans are homozygous for the SULT1A1*2 allele. These results are significant in that should these allele-specific responses also occur in tumors -- SULT1A1 genotype might be associated with clinical response to tamoxifen. That possibility will be explored in a clinical study as part of specific aim 3 of this grant.

REFERENCES

None.

Figure 1. SULT1A1 Activity toward E2 and OHT

■ 17β-estradiol (E2)
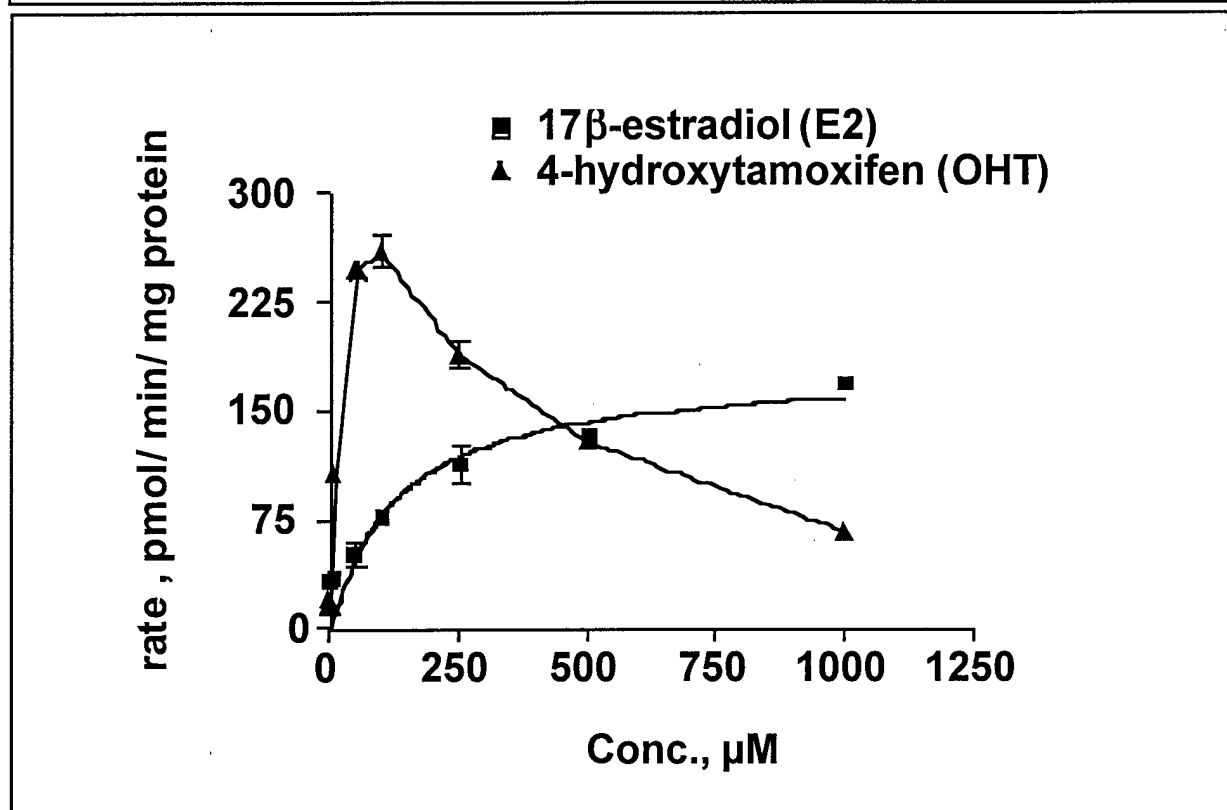▲ 4-hydroxytamoxifen (OHT)

Figure 1. 100 ng of purified rSULT1A1*1 was assayed for activity toward E2 and OHT in a standard radiometric SULT assay. SULT1A1 catalyzed the sulfation of both E2 and OHT.

Figure 2. Southern and Northern Analyses of MCF-7 Cell Lines

A. Southern Blot

B. Northern Blot

pCR3.1/ SULT1A1

— 2000 bp

— 1500 bp

pCR3.1

— 500 bp

SULT1A1

β-Actin

pCR3.1 Control

SULT1A1*1

SULT1A1*2

pCR3.1 Control

SULT1A1*1

SULT1A1*2

MCF-7 Cell Line Genomic DNA
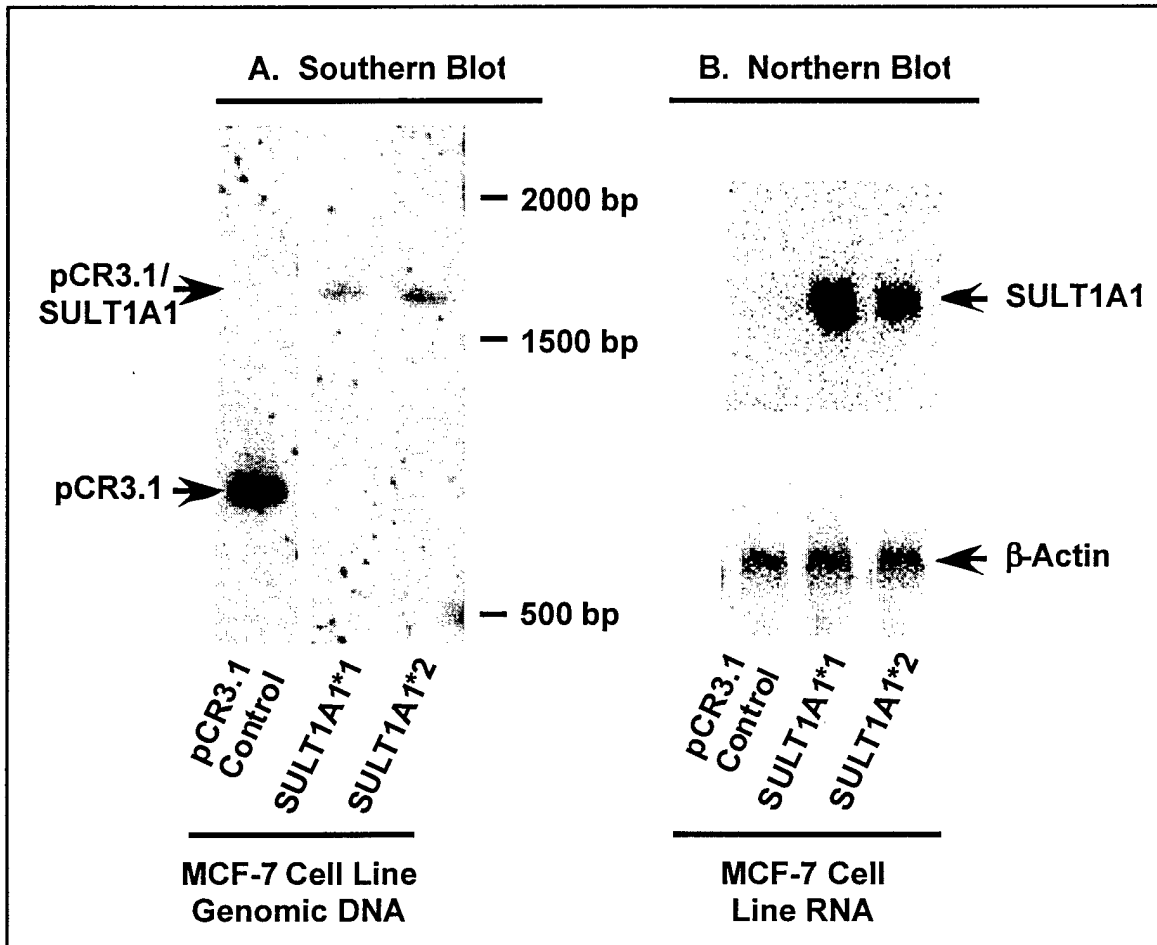
MCF-7 Cell Line RNA

Figure 2. A) Southern and B) Northern blot analyses of selected clones stably transfected with a pCR3.1 plasmid containing SULT1A1*1, *2 or no cDNA insert. Clones expressing SULT1A1 cDNAs had integrated similar gene copy numbers (A) and, relative to the pCR3.1 control expressed similar amounts of SULT1A1 mRNA (B). Expression of SULT1A1 mRNA was detectable in the pCR3.1 clone with longer X-ray film exposure
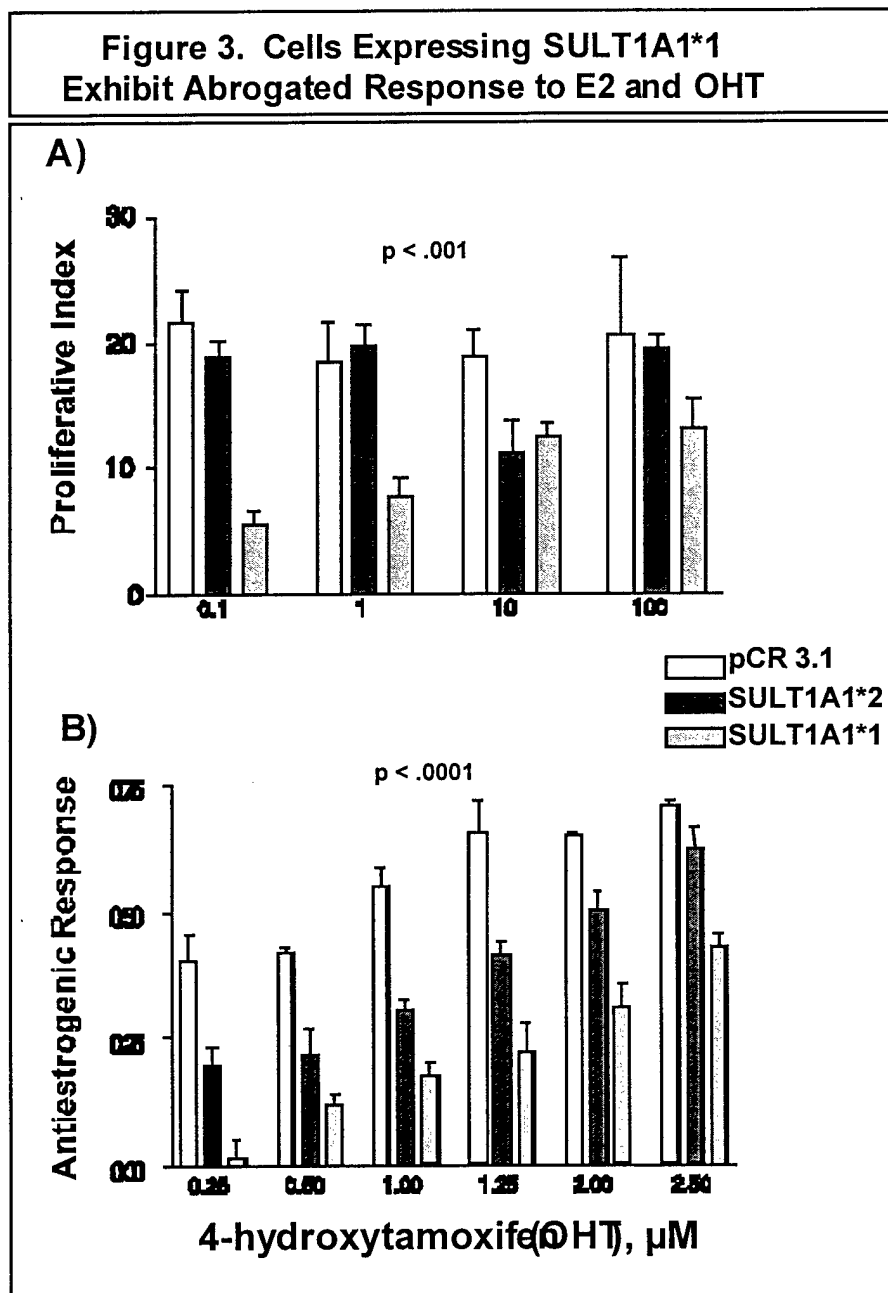
Figure 3. Cells Expressing SULT1A1*1 Exhibit Abrogated Response to E2 and OHT

A)

B)

4-hydroxytamoxifen (OHT), µM

Figure 3. Cells respond to E2 and OHT in a SULT1A1 allele-dependent manner. Panel A shows the proliferative response of control (pCR3.1), SULT1A1*1 expressing and SULT1A1*2 expressing cells to E2. Panel B depicts the antiestrogenic response of the same cells to OHT in the presence of 1 nM E2. Data represent the mean of three independent analyses. p values determined by ANOVA

Table 1. Common Nucleotide and Amino Acid Variations of the UGT1A6 First Exon in 65 Healthy Caucasians

Allele	Nucleotide (Amino Acid)								Allele Frequency (n=130 alleles)	
	19	(7)	315	(105)	541	(181)	552	(184)		
1	T	(Ser)	A	(Leu)	A	(Thr)	A	(Arg)	0.648	(85)
2	G	(Ala)	G	(Leu)	G	(Ala)	C	(Ser)	0.273	(35)
3	G	(Ala)	A	(Leu)	A	(Thr)	A	(Arg)	0.039	(5)
4	G	(Ala)	G	(Leu)	A	(Thr)	C	(Ser)	0.039	(5)

Allele frequencies were determined from a population of 65 healthy Caucasian subjects by either DNA sequencing or application of a PCR-RFLP assay.

Figure 4. UGT1A6 Genotype/Phenotype Analysis in Human Liver
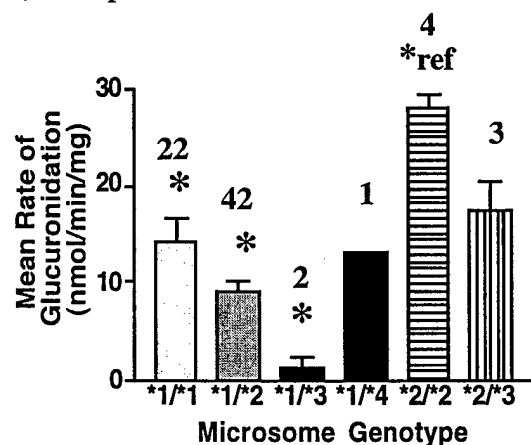
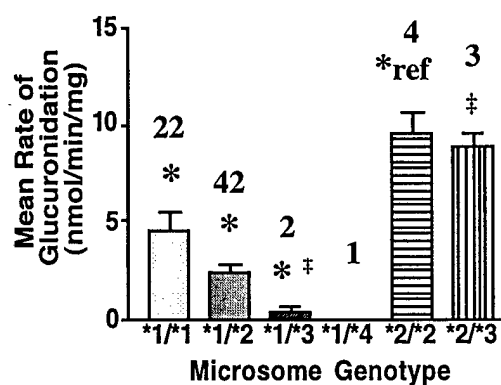A) α-Naphthol   B) p-Nitrophenol

Figure 4. Liver samples that were homozygous for UGT1A6*2 glucuronidated substrates at greater rates than samples that were homozygous for UGT1A6*1 or heterozygous for *1/*2 or *1/*3. Mean glucuronidation rates of α-naphthol (A) and p-nitrophenol (B) were determined by spectrophotometric analyses. The number of liver microsomes associated with each genotype are listed above each error bar. ANOVA analysis followed by Tukey's comparison test was performed. In both A and B, each of the asterisked bars represented significantly different mean glucuronidation rates for these genotyped samples from the mean rate of UGT1A6*2/*2 (*ref): *1/*1, *1/*3, and *1/*2 ($p < 0.05$, $p < 0.01$, $p < 0.001$, respectively). In B, the mean glucuronidation rate of p-nitrophenol by samples expressing *1/*3 was significantly different from samples expressing*2/*3 ($p < 0.05$)(‡).

·Rebecca B. Raftogianis

APPENDICES

1. Abstract: UGT1A6 Genetic Polymorphisms: Identification and Genotype/Phenotype Analysis from Human Liver Tissues, J.J. Zalatoris and R.B. Raftogianis, *Clin. Pharm. Ther.*, **69**:77, 2001. Presented (Poster) at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, March, 2001, Orlando, Fla.

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PIII-51

CEFAZOLIN ADMINISTRATION AND 2-METHYL-1,3,4-THIADIAZOLE-5-THIOL (MTD) IN HUMAN TISSUE. T.C. Wood, BA,[1]* K.L. Johnson, BS,[2]* S. Naylor, PhD,[1,2]* and R.M. Weinshilboum, MD,[1] Departments of Pharmacology and Experimental Therapeutics[1] and Biochemistry and Molecular Biology,[2] Mayo Clinic, Rochester, MN.

Cephalosporin antibiotic administration can lead to the formation of heterocyclic thiol metabolites that have been associated with hypoprothrombinemia and hemorrhage. The cefazolin structure includes a heterocyclic thiol, MTD, that can inhibit the γ-carboxylation of glutamate required to produce active clotting factors. We set out to determine whether MTD might be present in tissue from patients treated with cefazolin prior to clinically-indicated surgery. To test that hypothesis, we took advantage of the fact that heterocyclic thiols can be S-methylated by thiopurine methyltransferase (TPMT). As a first step, recombinant human TPMT was used to S-methylate MTD. MTD was a TPMT substrate with an apparent K_m of 63 µM. TPMT was then used to radioactively label a methyl acceptor substrate present in liver and kidney cytosol from patients treated preoperatively with cefazolin. Pooled renal cytosol was then used to isolate the methylated product by reverse-phase HPLC. The methylated product in kidney cytosol coeluted with methylated MTD during HPLC. When this methylated product was subjected to tandem mass spectroscopy, it was identified as S-methyl-MTD. MTD, an inhibitor of clotting factor activation, is present in the tissues of patients treated with cefazolin.

PIII-52

HUMAN 3'-PHOSPHOADENOSINE-5'-PHOSPHOSULFATE SYNTHETASE2 (PAPSS2) PHARMACOGENETICS: GENE CLONING, RESEQUENCING AND SINGLE NUCLEOTIDE POLYMORPHISMS, (SNPs). Z-H. Xu, MD,* R.R. Freimuth, BA,* B. Eckloff, BA,* E. Wieben, PhD,* and R.M. Weinshilboum, MD, Mayo Clinic, Mayo Foundation, Rochester, MN.

PAPS is the co-substrate for all sulfotransferase (SULT) enzymes. These enzymes catalyze the sulfate conjugation of many endogenous and exogenous compounds, including drugs and other xenobiotics. In humans, PAPS is synthesized from ATP and $SO_4{}^{2-}$ by two bifunctional PAPSS isoforms. A rare inactivating mutation within PAPSS2 results in major skeletal deformity in humans. To determine whether less striking "pharmacogenomic" variations in the PAPSS2 gene might be one factor responsible for individual differences in sulfate conjugation, we cloned *PAPSS2* and "resequenced" all exons and splice junctions, as well as the core promoter using 90 Coriell Polymorphism Discovery Resource DNA samples. A total of 1 Mb of sequence was analyzed. Twenty-two SNPs were seen, including 4 non-synonymous cSNPs which altered the following amino acids: Glu10Lys, Met281Leu, Val291Met, and Arg432Lys. We also observed 4 insertions/deletions, and one sample was homozygous for an 81 bp deletion in the core promoter region. The functional significance of these polymorphisms will now be assessed by site-directed mutagenesis and transient expression.

PIII-53

UGT1A6 GENETIC POLYMORPHISMS: IDENTIFICATION AND GENOTYPE/PHENOTYPE ANALYSIS FROM HUMAN LIVER TISSUES. J.J. Zalatoris, PhD,* and R.B. Raftogianis,PhD, Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA.

UDP glucuronosyltransferase (UGT) enzymes catalyze the glucuronidation of endogenous and exogenous molecules. UGT1A6 is expressed in several tissues, including the liver, and metabolizes small phenolic molecules. We set out to identify UGT1A6 alleles in a Caucasian population and to determine the genotype/phenotype correlation of those alleles. We have identified three common single nucleotide polymorphisms (SNPs) in the first exon of UGT1A6 that alter encoded amino acids Ser7, Thr181 and Arg184. Different permutations of those three SNPs define four common alleles with frequencies of 0.69, 0.28, 0.022 and 0.011. SNP-specific RFLP assays were developed and applied to genotype DNA isolated from 43 random blood donors, 4 normal and 55 tumor liver tissues. Allele distribution followed the Hardy-Weinberg (HW) theorem in the random blood donors and normal liver tissue. Genotypes derived from liver tumors deviated from HW equilibrium such that the *1/*2 genotype was identified at a greater frequency than predicted. A spectrophotometric assay was developed to measure the rate of glucuronidation of naphthol and p-nitrophenol by UGT1A6. Microsomes prepared from the liver tissues were assayed to establish UGT1A6 phenotype. The most frequent genotypes, *1/*1 and *1/*2, were not associated with significantly different glucuronidation rates within liver tissues.

PIII-54

PHARMACOLOGIC INTERACTIONS BETWEEN TRANSDERMAL SELEGILINE AND COCAINE. J. Mendelson, MD, A.S. Dearborn, MS,* N. Uemura, MD,* L. Lester, AB,* N. Chiang, PhD, P. Jacob III, PhD,* and R.T. Jones, MD,* Drug Dependence Research Center, University of California, San Francisco, CA.

Selegiline may be efficacious in treatment of cocaine addiction. This study assessed pharmacokinetic (PK) and pharmacodynamic (PD) interactions between transdermal (TD) selegiline and cocaine in 14 nondependent, cocaine-experienced volunteers challenged with IV cocaine-d_5 before and after TD selegiline. To obtain steady state conditions for cocaine rapidly, a 0.5 mg/kg loading dose (over 10 min) was followed by a 4 hr infusion of 2.0 mg/kg. Transdermal selegiline was administered as one 20 mg/patch/day (Somerset Pharmaceuticals) applied after the first cocaine infusion and continued for 10 days. The second cocaine challenge was performed following 7 days of selegiline, at selegiline steady-state (determined by urine PK analysis). PD effects of cocaine (heart rate, blood pressure, respiratory rate, skin and tympanic temperature) were not altered by selegiline. Adverse reactions to TD selegiline were minimal with no orthostatic vital sign changes and no adverse cardiovascular reactions. Selegiline and its levo-metabolites had all reached steady-state after approximately 5 days. Plasma cocaine PK was unaffected by selegiline administration. These results suggest that TD selegiline is safe and may be useful in the treatment of cocaine dependence. Supported by NIDA contract N01DA-4-8306 and NIH RR-00079 (GCRC, UCSF).

OIII-A-1

IN VITRO CHARACTERIZATION OF NEW CYP2D6 ALLELIC VARIANTS. K.A. Marcucci, BS, T. Walker,* R.R. Gotschall, MS, R.E. Pearce, PhD,* I. Hurwitz, PhD,* J.S. Leeder, PhD, PharmD, and A. Gaedigk, MS, PhD, Division of Clinical Pharmacology and Toxicology, The Children's Mercy Hospital and Clinics, Kansas City, MO.

Two new mutations of the CYP2D6*17 allele have been identified in African Americans. A G1747C mutation was found on the 17 allele, alone (*17-a) or in combination with G3271A (*17-b). To characterize the functional consequences of the corresponding amino acid changes V136I and V338M, respectively, each was introduced by site directed mutagenesis into a *17 plasmid to generate constructs for heterologous expression in COS-7 cells. Expressed microsomal 2D6*1, *2, *17, *17-a and *17-b protein was analyzed for activity with dextromethorphan (DM) and bufuralol (BF) as substrates. Immunoblot analysis allowed for quantitation of the 2D6 proteins. Km (μM) and intrinsic clearance (CLint in μl/min/pmol) were determined in microsomes from n=2 transfection experiments.

CLint	*1	*2	*17	*17-a	*17-b
DM	0.024	0.017	0.004	0.002	0.002
BF	0.089	0.097	0.024	0.067	0.048

The notable difference in CLint between DM and BF suggest that activity conferred by the 2D6 isoforms is substrate dependent. Preliminary results suggest that the effects of the amino acid changes in all three *17 allelic variants appear to be more pronounced for DM than BF. To further investigate substrate dependency, Km and CLint will also be determined for sparteine and debrisoquine. The use of this consistent expression system (COS-7) will allow us to clearly define the *in vitro* metabolic consequences of 2D6 allelic variants.

OIII-A-2

NOVEL CYP2D6 ALLELES IN AFRICAN AMERICANS. A. Gaedigk, MS, PhD,[1] J.S. Leeder, PharmD, PhD,[1] and L.D. Bradford, PhD,[2]* Division of Clinical Pharmacology and Toxicology, The Children's Mercy Hospital and Clinics, Kansas City, MO[1] and The Clinical Pharmacology Research Unit, Morehouse School of Medicine, Atlanta, GA.[2]

A large well defined African American (AA) population (n=254) has been phenotyped with the CYP2D6 probe dextromethorphan (DM) and/or genotyped for 18 allelic variants. The allele frequencies were distinct between Caucasians, Asians and Black Africans. The presence of *17 (f=0.2) appears to be partially responsible for the pronounced 'right shift' observed in the activity distribution. 2D6*7, *8, *11, *15 and *18 were not found in AA. Eleven/254 subjects had PM phenotypes (DM/DX>0.3) and EM genotypes. To find novel allelic variants we cloned and sequenced both alleles from a *2/*17 PM subject. SNPs at positions 1747 (homozygous) and 3271 (heterozygous) were detected revising the genotype to *29/*17-a. PCR-RFLP based genotyping assays were developed and SNPs at 1747 and 3271 were only found in genotypes having at least one *2 or *17 allele. Since SNPs at 1747 and 3271 were discovered in one subject genotyped as *1/*17, they appear to be linked not only with *29, but also with *17 (*17-b). In total, 36 alleles (f=0.07) were positive for both SNPs, and 29 were revised from *2 to *29. The remaining 7 subjects carried the SNPs either on their *2 or *17 allele and further tests are needed for discrimination. *In vitro* evaluation of the functional consequences of the 1747/3271 SNPs towards substrates used as phenotyping probes (DM, bufuralol, debrisoquine, sparteine) will further clarify the genotype-phenotype discordance observed *in vivo*.

OIII-A-3

HUMAN SULFOTRANSFERASE (SULT) 2A1 PHARMACOGENETICS: GENOTYPE TO PHENOTYPE STUDIES. B.A. Thomae, BS,* B. Eckloff, BA,* R.R. Freimuth, BA,* E. Carlini, PhD,* E. Wieben, PhD,* and R.M. Weinshilboum, MD, Mayo Clinic-Mayo Foundation, Rochester, MN.

Sulfate conjugation catalyzed by cytosolic SULTs plays an important role in the metabolism of many drugs, other xenobiotics and hormones. SULT2A1 catalyzes the sulfate conjugation of dehydroepiandrosterone (DHEA) as well as other steroids and is expressed in the liver, small intestine and adrenal cortex. We set out to determine whether there might be common, functionally significant single nucleotide polymorphisms (SNPs) in the human SULT2A1 gene. As a first step, we "resequenced" 60 DNA samples from African American and 60 samples from Caucasian subjects. All 6 exons as well as a portion of the 5'-flanking region were sequenced with dye primer chemistry, for a total of approximately 0.6 Mb of sequence. We observed 15 novel SNPs, including 3 non-synonymous cSNPs detected only in African Americans. Expression constructs were created that included all of the nonsynonymous cSNPs observed and were expressed in COS-1 cells. After correction for transfection efficiency, there was a significant decrease in SULT2A1 activity for all three constructs that included SNPs as compared to that of the "wild type" sequence. These observations raise the possibility of ethnic-specific pharmacogenetic variation in SULT2A1-catalyzed sulfation.

OIII-A-4

HUMAN SULT1A1 PHARMACOGENETICS: ASSOCIATION OF SULT1A1*2 WITH HEIGHTENED CELLULAR RESPONSE TO ESTROGENS AND ANTIESTROGENS. R.B. Raftogianis, PhD and S.E. Walther,* Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA.

In breast tissue, SULT1A1-mediated sulfation of 4-hydroxytamoxifen (OHT), an antiestrogen, and 17β-estradiol (E_2) results in the biological inactivation of these molecules. We have previously shown that the SULT1A1*2 variant allele, which encodes an Arg213His polymorphism, exhibits significantly lower SULT1A1 activity in human platelet samples as compared to the *1 allozyme. To further characterize their biological differences, recombinant SULT1A1*1 and *2 proteins were generated using the baculovirus expression system. The *2 protein exhibited a 20-fold lower maximal activity toward OHT and p-nitrophenol as compared to *1. Pulse-chase experiments in infected sf-9 cells indicated that the *1 and *2 proteins were stable for up to 24 hours but the *2 variant may be targeted for degradation by the ubiquitin pathway, accounting for a 5-10 fold decrease in the steady-state level of the *2 protein. Stably transfected MCF-7 cell lines were generated to overexpress SULT1A1*1 and *2 proteins. Cells expressing *1 proliferated at a lower rate in response to E_2 (p=0.01) and at a significantly higher rate in the presence of OHT (p=0.003) as compared to control cells and those expressing SULT1A1*2. These results suggest that the *2 allele might predispose cells to the mitogenic effects of estrogens but also render them more susceptible to the antiestrogenic effects of OHT.

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Human Phenol Sulfotransferase (SULT1A1) Pharmacogenetics and Regulation of Cellular Response to 17β-estradiol and 4-hydroxytamoxifen.

Rebecca B Raftogianis, Susan E Walther, Fox Chase Cancer Center, Philadelphia, PA.

Sulfate conjugation is a metabolic pathway that inactivates estrogens such as 17β-estradiol (E2) and the antiestrogen 4-hydroxytamoxifen (OHT). A phenol sulfotransferase, SULT1A1, catalyzes those sulfation reactions in several human tissues including the breast. We had previously identified a common genetic polymorphism in the human SULT1A1 gene (SULT1A1*2) that encoded a single amino acid change, Arg213His. The *2 protein was associated with a significantly lower capacity to sulfate a prototypic substrate, p-nitrophenol, as compared to the *1 protein using in vitro biochemical assays. The studies presented here were undertaken to determine the significance of the SULT1A1*2 polymorphism in cellular response to two biologically relevant substrates, E2 and OHT, in a cell model system. MCF-7 cells were stably transfected with SULT1A1*1, SULT1A1*2 or mock transfected with the pCR3.1 expression vector. 1A1*1 and *2 cell lines were chosen for further study based upon equal level of SULT1A1 mRNA expression. All three cell lines (1A1*1, *2 and pCR3.1) were evaluated for proliferative response to treatment with E2 and, separately, OHT using the alamarBLUE metabolic assay. The cells expressing SULT1A1*2 responded identical to the mock transfected cells, but the cells expressing SULT1A1*1 exhibited an abrogated response (p<0.0001) over a wide range of E2 concentrations. Cells were subsequently exposed to varying concentrations of OHT in the presence of a fixed E2 concentration (1 nM). The mock transfected cells exhibited the greatest response to OHT, followed by the cells expressing 1A1*2 while the cells expressing 1A1*1 exhibited a relative lack of response to OHT (p < 0.0001). Subsequent characterization of protein stability, using pulse-chase analyses, suggested that the SULT1A1*2 protein was three-fold less stable than the wildtype protein. Western and Northern blot analyses determine that within the context of equal level of SULT1A1 mRNA expression, the steady state level of SULT1A1 protein was lower in cells expressing the *2 variant than in cells expressing the *1 allele. These data suggest that individuals who are homozygous for the SULT1A1* 2 allele (about 10% of Caucasian and African American populations) might be most susceptible to the mitogenic effects of E2 but also more responsive to tamoxifen therapy.

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